蝶と蛾　*Lepidoptera Science* **69**（3/4）: 93−98, December 2018

Discovery of *Blastobasis spiniella*（Lepidoptera: Blastobasidae）in Japan

Issei Ohshima[1]*, Sadahisa Yagi[2] and David Adamski[3]

[1]Department of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan
[2]Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan
[3]Department of Entomology, National Museum of Natural History: E-526, MRC-168; P.O. Box 37012, Smithsonian Institution, Wash., DC USA 20013-7012.

Abstract　*Blastobasis spiniella* Park, 2000 is newly recorded from Japan. The habitus of *B. spiniella* is presented, and male and female genitalia are shown. A phylogenetic tree is inferred based on COI barcode sequences of *B. spiniella* and other known *Blastobasis* species from Japan.

Key words　*B. inouei*, *B. sprotundalis*, COI, DNA barcoding, female genitalia, new record.

Introduction

Blastobasis Zeller, 1855 is the largest genus in the family Blastobasidae, consisting of nearly 140 described species（Sinev, 2014）. In Japan, two *Blastobasis* species have been reported: *B. sprotundalis* Park, 1984 and *B. inouei* Moriuti, 1987（Moriuti, 1987）. Although the distribution of *B. inouei* appears to be restricted to Hokkaido Island, *B. sprotundalis* has a wider distributional range, including Hokkaido, Honshu, Shikoku and Kyushu Islands in Japan, as well as South Korea and Primorsky Krai in Russia.

Recently, we had the opportunity to examine blastobasid specimens collected at the Hikosan Biological Laboratory（HBL）（Fig. 1）, Kyushu University in Fukuoka, Kyushu, Japan. During the identification process, we found two specimens of *B. spiniella*. This species was originally described from South Korea（Park, 2000）, and it is presently described from Japan for the first time, outside of its type locality.In this paper, we newly record *Blastobasis spiniella* from Japan and show its female genitalia for the first time. We also show hypothesized relationships for sequences of the COI barcoding region of the two *B. spiniella* specimens with the same sequences from specimens of *B. inouei* and *B. sprotundalis*, as well as other species collected from Japan.

Materials and methods

1. Sampling

All samples were collected by light trap using mercury vapor lamp, except for IO-306, which was reared from a larva（Table 1）. Sampling was conducted at HBL between 2014 and 2016. Additional *Blastobasis* spp.（*B. sprotundalis* and *B. inouei*）and other blastobasid taxa（*Neoblastobasis biceratala* and *Lateantenna decolor*）were collected from various Japanese localities between 2001 and 2016（Table 1）. These additional taxa were used to compare barcode sequence data and for phylogenetic analyses.

2. DNA extraction, COI barcoding and phylogenetic analyses

All specimens used in this study（Table 1）were targeted for DNA extraction, except for a single *B. inouei* female（IsO-73）. DNA was extracted from the abdomen of each specimen using DNeasy Blood & Tissue Kit（Qiagen）following manufacturer's protocol.

We amplified a barcoding region of the mitochondrial COI gene（658 bp）using a primer set LCO1490 + HCO2198（Folmer *et al*., 1994）. Subsequent PCR, purification and sequencing procedures were as described by Ohshima *et al*.（2018）. The obtained sequences were aligned manually without ambiguity or indels using Mesquite（Maddison and Maddison, 2016）. Pairwise sequence distances were calculated using PAUP* 4.0a159 for Macintosh（Swofford, 2002）based on the Kimura-two-parameter（K2P）model.

For phylogenetic analyses, maximum parsimony（MP）and maximum likelihood（ML）analyses were conducted using PAUP. All characters were equally weighted in the

*Corresponding author E-mail: issei@kpu.ac.jp

Fig. 1. Sampling site at the Hikosan Biological Laboratory (HBL), Kyushu University. (A) Forest around HBL, consisting of deciduous and evergreen vegetation, dominated by *Zelkova serrata, Acer pictum, Camellia japonica* and *Cinnamomum tenuifolium*; (B) Setup for light trapping.

Table 1. Sample information used in the present study.

Species name	DNA extraction number	Genitalia slide number	Specimen condition	Sex	Sampling locality and number	Sampling date	GenBank accession number
Blastobasis spiniella	IY-559	IY-559	Dried	Male	HBL, Fukuoka, Japan	7, Aug, 2014	LC427101
Blastobasis spiniella	IY-596	IY-596	Dried	Female	HBL, Fukuoka, Japan	27, Jul, 2015	LC427102
Blastobasis inouei	IO-312	IO-312	Stored in 99.5% ethanol	Male	Shibetsu, Hokkaido, Japan (Bottle no. IsO-137)	14, Aug, 2001	LC427103
Blastobasis inouei	N/A	IsO-73	Dried	Female	Kuriyama, Hokkaido, Japan	19, Jul, 1995	N/A
Blastobasis sprotundalis	IO-252	IO-252	Dried	Female	Sendai, Miyagi, Japan	12-13, Jul, 2002	LC427105
Blastobasis sprotundalis	IO-253	IO-253	Dried	Male	Sendai, Miyagi, Japan	12-13, Jul, 2002	LC427106
Blastobasis sprotundalis	IO-255	IO-255	Dried	Male	Sendai, Miyagi, Japan	12-13, Jul, 2002	LC427108
Blastobasis sprotundalis	IO-258	IO-258	Dried	Male	HBL, Fukuoka, Japan	3, Jul, 2016	LC427107
Blastobasis sprotundalis	IY-592	IY-592	Dried	Female	HBL, Fukuoka, Japan	25, Jul, 2014	LC427104
Blastobasis sprotundalis	IO-308	IO-308	Dried	Male	Tanegashima, Kagoshima, Japan	2, Aug, 2016	LC427109
Neoblastobasis biceratala	IO-261	IO-261	Dried	Female	HBL, Fukuoka, Japan	3, Jul, 2016	LC427110
Lateantenna decolor	IO-306	IO-306	Alive	Female	Higashihiroshima, Hiroshima, Japan	4, Aug, 2016	LC311233

N/A, not available.

Table 2. Intra- and interspecific pairwise K2P distances in the COI barcoding region among studied samples.

		1	2	3	4	5	6	7	8	9	10	11
1	*B. spiniella* (IY-559, HBL)	–										
2	*B. spiniella* (IY-596, HBL)	0.00457	–									
3	*B. inouei* (IO-312, Shibetsu)	0.02959	0.02483	–								
4	*B. sprotundalis* (IY-592, HBL)	0.07348	0.06844	0.06349	–							
5	*B. sprotundalis* (IO-252, Sendai)	0.07181	0.06678	0.06184	0.00152	–						
6	*B. sprotundalis* (IO-253, Sendai)	0.07181	0.06678	0.06184	0.00152	0	–					
7	*B. sprotundalis* (IO-258, HBL)	0.07181	0.06678	0.06184	0.00152	0	0	–				
8	*B. sprotundalis* (IO-255, Sendai)	0.07012	0.06511	0.06018	0.00305	0.00152	0.00152	0.00152	–			
9	*B. sprotundalis* (IO-308, Tanegashima)	0.06847	0.06681	0.05523	0.01539	0.01385	0.01385	0.01385	0.01229	–		
10	*N. biceratala* (IO-261, HBL)	0.08897	0.0838	0.07688	0.07844	0.07846	0.07846	0.07846	0.07676	0.0836	–	
11	*L. decolor* (IO-306, Higashihiroshima)	0.09227	0.08709	0.08202	0.09382	0.09211	0.09211	0.09211	0.09038	0.10081	0.09052	–

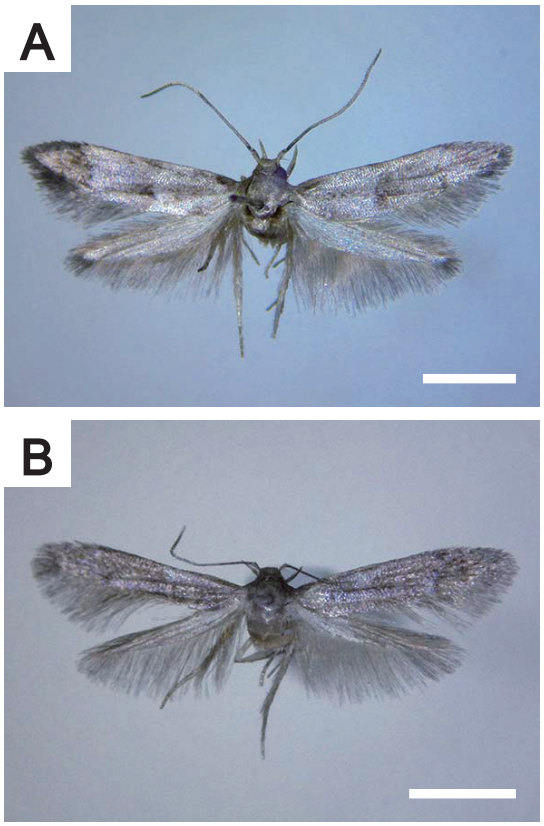

Fig. 2. Adults of *Blastobasis spiniella* Park. (A) Male (DNA extraction and genitalia slide number IY-559); (B) Female (DNA extraction and genitalia slide number IY-596). Scale bar: 2 mm.

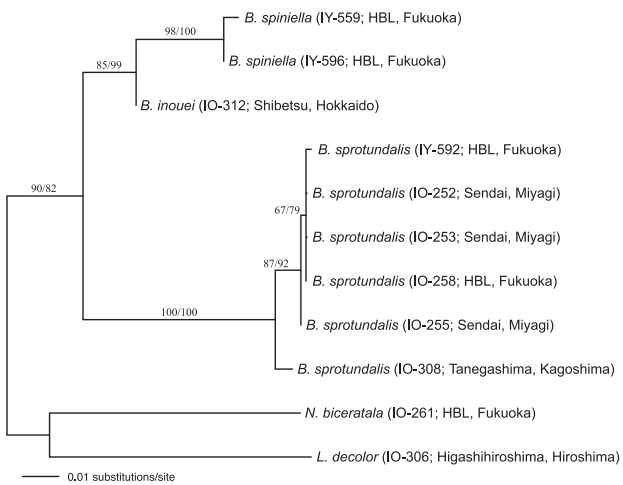

Fig. 3. A ML tree based on the COI sequences. Branch lengths are proportional to ML estimated genetic distances. Numbers associated with branches indicate bootstrap values higher than 50% (ML/MP). Within sample names, species names are followed by each DNA extraction number and sampling locality.

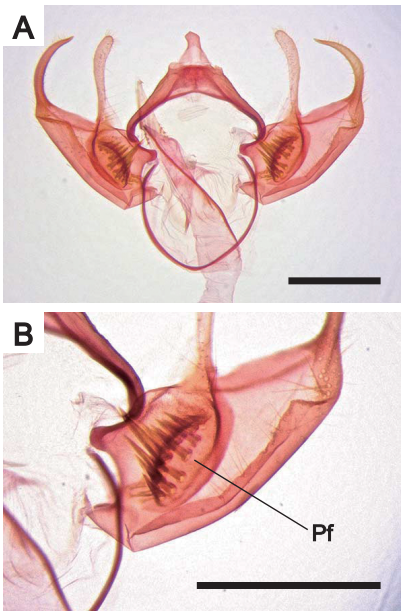

Fig. 4. Male genitalia of *Blastobasis spiniella* Park (DNA extraction and genitalia slide number IY-559). (A) Total genitalia; (B) Enlarged proximal flange of right valva. Pf, proximal flange. Scale bar: 0.2 mm.

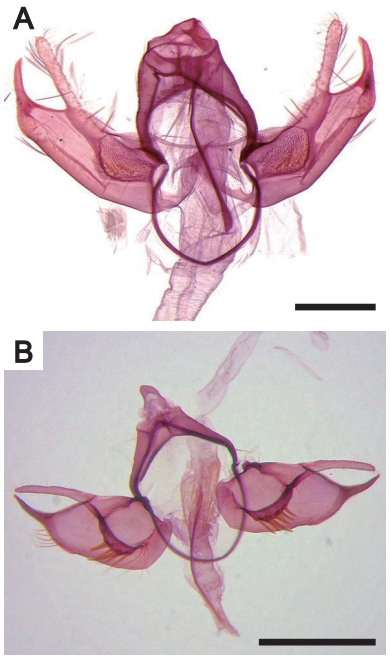

Fig. 5. Male genitalia of other two Japanese *Blastobasis* species. (A) *Blastobasis sprotundalis* (DNA extraction and genitalia slide number IO-258); (B) *Blastobasis inouei* (DNA extraction and genitalia slide number IO-312). Scale bar: 0.2 mm.

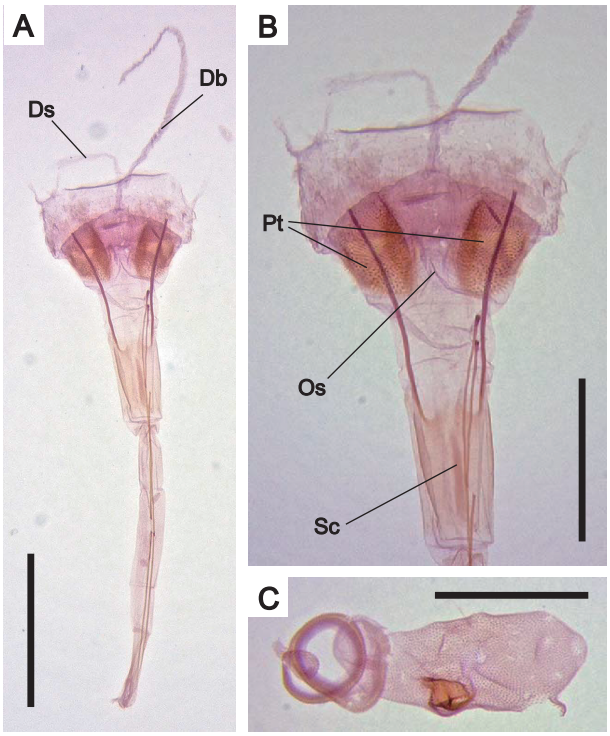

Fig. 6. Female genitalia of *Blastobasis spiniella* Park (DNA extraction and genitalia slide number IY-596). (A) Distal part of genitalia without corpus bursae; (B) Enlarged seventh segment, intersegmental membrane between seventh and eighth segments, and eighth segment; (C) Corpus bursae. Db, ductus bursae; Ds, ductus seminalis; Os, ostium bursae; Pt, densely microtrichiate patches on seventh and eighth intersegmental membrane; Sc, sclerotized streak along median longitudinal axis of eighth abdominal tergite. Scale bars: 1 mm in A and 0.5 mm in B and C.

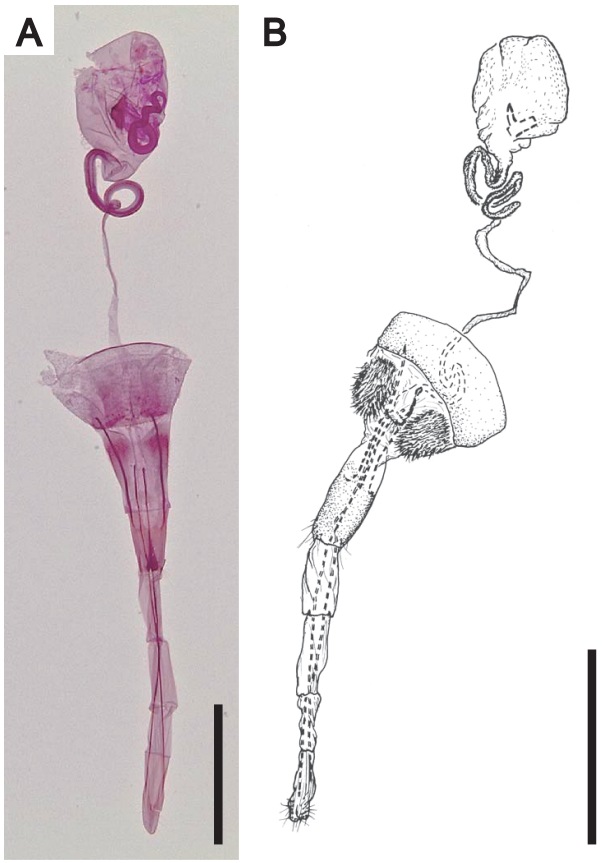

Fig. 7. Female genitalia of two other Japanese *Blastobasis* species. (A) *Blastobasis sprotundalis* (DNA extraction and genitalia slide number IY-592); (B) *Blastobasis inouei* (genitalia slide number IsO-73; collected in Kuriyama, Hokkaido, Japan on 19, July, 1995). Scale bar: 1 mm.

MP analysis, and MP trees were searched by 100 random addition replications with tree bisection reconnection (TBR) branch swapping. The best model of nucleotide substitutions for the ML analysis was selected using the Automated Model Selection option in PAUP based on the Akaike Information Criterion (AIC) (Akaike, 1974), and the GTR+I model (unequal base frequencies: A = 0.29328153, C = 0.14586035, G = 0.14614348, T = 0.41471464 ; six substitution rate categories: A–C = 10.177825, A–G = 11.651884, A–T = 13.35307, C–G = 2.5498437, C–T = 65.356711, G–T = 1 ; proportion of invariable sites = 0.776817) was selected. ML trees were searched with TBR branch swapping using neighbor-joining tree as a starting point. To assess confidence in clades, bootstrap tests (Felsenstein, 1985) were performed using 100 replicates with TBR branch swapping for both MP and ML analyses. NEXUS files of the aligned sequences are available from http://eureka.kpu.ac.jp/~issei/Ohshima_Lab/Data.html or by request to the first author.

3. Morphological investigation

Abdomens used for DNA extraction were placed in 10% KOH solution following extraction and kept at 60℃ for approximately 10 min to clean residual scales and internal soft tissues. The remaining abdominal exoskeleton and genitalia were stained with aceto-fuchsin and mounted on a microscope slide in Euparal. Adult wing patterns were examined using a Leica S6D stereoscopic microscope and genital morphology was observed using Leica M205C and DM2500 stereoscopic microscopes. All specimens were deposited in the collection of the Entomology Laboratory of Kyoto Prefectural University, Kyoto, Japan.

Results and Discussion

1. Sequence comparison and phylogenetic analyses

A single male specimen (IY-559 ; Fig. 2A), which was identified as *B. spiniella* by external genital morphology (Fig. 4), showed 3 bp differences (K2P distance, 0.46%) from a female specimen (IY-596 ; Fig. 2B) in the sequenced 658 bp COI barcoding region (Table 2). This pairwise distance was much smaller than the maximum intraspecific pairwise distance in *B. sprotundalis* (1.54%) (Table 2), indicating that IY-596 is a female of *B. spiniella*. The two *B. spiniella* specimens showed 2.96% (IY-559) and 2.48% (IY-596) pairwise distances from the *B. inouei* specimen (IO-312), and these interspecific distances were slightly smaller than the empirical interspecific distances (e.g., Hebert *et al*., 2003, where 98% of assessed 200 lepidopteran species showed > 3% interspecific distances). The distances between *B. spiniella* and *B. inouei* were smaller than those between *B. spiniella* and *B. sprotundalis* (6.51–7.35%) and between *B. inouei* and *B. sprotundalis* (5.52 – 6.35%) (Table 2). However, the interspecific distances between *B. spiniella* and *B. inouei* were larger than the intraspecific distances in *B. spiniella* (0.46%) and *B. sprotundalis* (0 – 1.54%). These observations, coupled with morphological differences in genital characters between *B. spiniella* and *B. inouei* (Figs. 4, 5), confirm that *B. spiniella* and *B. inouei* are separate species.

Phylogenetic analyses yielded trees with identical topology in both the ML and MP analyses. The *Blastobasis* specimens collected at HBL (Table 1) were clearly divided into two clades (Fig. 3). The two *B. spiniella* specimens formed a monophyletic group with *B. inouei* (IO-312), with high bootstrap values (ML 85%; MP 99%). This is consistent with the morphological similarity between the two species. The second *Blastobasis* clade consisted of *B. sprotundalis*, and monophyly of the *B. sprotundalis* clade was strongly supported with bootstrap values (ML 100%; MP 100%). Although all sequenced *Blastobasis* specimens formed a monophyletic group with high bootstrap support values (ML 90%; MP 82%), we do not presume the monophyly of the genus *Blastobasis* because our sample size was too small. Adamski (unpublished results) found *Blastobasis* to be paraphyletic using macromorphological data.

2. Description

Blastobasis spiniella Park, 2000 (Figs. 2, 4, 6).

Blastobasis spiniella Park, 2000 : 245 – 246 , Fig. 1 A (adult), Fig. 2A (wing venation), B (antennal notch), C (male genitalia), and D (phallus).

Material examined. 1♂1♀: Kyushu– 1♂, Hikosan, Tagawa, Fukuoka Pref., 7 viii 2014, Sadahisa Yagi leg., DNA extraction and genitalia slide number IY-559 ; 1♀, Mt. Hiko 670 m, Tagawa, Fukuoka Pref., 27 vii 2015, Sadahisa Yagi leg., DNA extraction and genitalia slide number IY-596.

Diagnosis. *Blastobasis spiniella* is similar to *B. sprotundalis* and *B. inouei* in wing pattern (Fig. 2) but differs from *B. sprotundalis* (Fig. 5A) by having a large cluster of long spine-like setae on the proximal flange of the valva (Fig. 4B) and from *B. inouei* (Fig. 5 B) by having a distinctively long bristle seta on the proximal flange (Fig. 4B). *B. spiniella* female genitalia differs from that of *B. sprotundalis* (Fig. 7A) by having a pair of large densely microtrichiate patches on the intersegmental membrane between the seventh and eighth sterna, juxtaposed to the ostium and by lacking a darkly pigmented triangular streak on the eighth sternum (Fig. 6A, B). Females of *Blastobasis spiniella* differ from females of *B. inouei* (Fig. 7B) by having a densely spiculate corpus bursae (Fig. 6C).

Wing expanse. ♂, 11.8 mm (IY-559) (Fig. 2A); ♀, 9.5 mm (IY-596) (Fig. 2B).

Female genitalia (Fig. 6). Ovipositor telescopic, with four membranous subdivisions. Eighth abdominal tergum with a narrow, darkly pigmented streak along the median longitudinal axis. Apophysis posterioris approximately three times as long as apophysis anterioris. Intersegmental membrane between seventh and eighth sterna with a pair of large densely microtrichiate patches, juxtaposed to ostium bursae. Seventh sternum straight along posterior margin. Ductus bursae narrow, posterior part membranous; anterior part coiled, with two rows of overlapping platelets; ductus seminalis arising near anterior margin of seventh sternum. Corpus bursae elliptical, densely spiculate; signum, a hornlike process arising from an angular base.

Distribution. Japan (Kyushu) (new record), South Korea (Park, 2000).

Acknowledgments

We thank T. Arai, H. Inoue, U. Jinbo, M. Kimura and K.

Sugisima for collecting the blastobasid samples, T. Hirowatari and Y. Nasu for arranging a field trip to Hikosan in 2016 , K. Mizota for his assistance with the field collection in Sendai and I. Yamamoto, who was supported by a Research Support Staff grant from Kyoto Prefectural University, for his assistance with DNA sequencing.

References

Akaike, H., 1974. A new look at the statistical model identification. *IEEE Trans. Automat. Control* **19**: 716-723.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek, 1994. DNA primers for amplification of mitochondrial Cytochrome C Oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**: 294-299.

Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard, 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. B* **270**: 313-321.

Maddison, W.P. and D.R. Maddison, 2016. Mesquite: a modular system for evolutionary analysis. Version 3.10. http://mesquiteproject.org

Moriuti, S., 1987. Records and descriptions of Blastobasidae (Lepidoptera) from Japan. *Tinea* **12**, (Suppl): 168-181.

Ohshima, I., Y. Sakamaki, H. Inoue, T. Arai and D. Adamski, 2018. DNA barcoding and adult morphology reveal an unrecorded species on *Citrus* and other new host associations of Blastobasidae (Lepidoptera: Gelechioidea) in Japan, with taxonomic notes on the genus *Lateantenna*. *Lepid. Sci*. **69**: 1-9.

Park, K.-T., 2000. New records of Blastobasidae (Lepidoptera) from Korea, with description of a new species. *Korean J. Biol. Sci*. **4**: 245-250.

Sinev, S.Y., 2014. World catalogue of blastobasid moths (Lepidoptera, Blastobasidae). ZIN RAS. 108 pp. St. Petersburg. (in Russian)

Swofford, D.L., 2002. *PAUP*. Phylogenetic Analysis Using Parsimony* (**and Other Methods*). Version 4. Sinauer Associates, Sunderland, Massachusetts.

摘　要

Discovery of *Blastobasis spiniella* (Lepidoptera: Blastobasidae) in Japan

スピニエラネマルハキバガ(新称)(鱗翅目：キバガ上科：ネマルハキバガ科)の日本からの発見(大島一正・屋宜禎央・D. Adamski)

九州大学農学部附属英彦山生物学実験施設で採集されたネマルハキバガ科の標本を検討したところ，日本未記録で，かつこれまで韓国からしか記録のなかった *Blastobasis spiniella* Park, 2000 を確認した．本種はこれまでオス個体しか知られていなかったため，まず交尾器形態を原記載と比較し，*B. spiniella* のオス 1 個体を同定した．次にこの個体の COI バーコーディング領域の配列を他標本の配列と比較することで，*B. spiniella* のメス 1 個体を特定した．

Blastobasis spiniella Park, 2000 スピニエラネマルハキバガ（新称）

開張，オス 11.8 mm，メス 9.5 mm．斑紋では *Blastobasis* 属の日本産 2 既知種（*B. sprotundalis* と *B. inouei*）との区別は難しいが，オス把握器基部のプロキシマルフレンジに多数の棘状刺毛があり，かつ，棘状刺毛のうち中央部の 1 本が他に比べて顕著に長いことでこれら 2 既知種とは区別される．メス交尾器では，腹部第 7-8 節間膜質部の交尾口の両側に微細な刺毛がパッチ状に多数見られ，かつ，交尾嚢に多数の篏刻が見られることで上記 2 種とは区別が可能である．

(Received May 3, 2018. Accepted June 24, 2018)